REMARKS

Upon entry of this amendment, claims 1, 5-12, 14-17, 19-20, 77-79, 81-85, 88, 90-94, 101-110, 112-114, 116-120, 122-127, 130-137, 139-148, 150-159, 161-174, 176-190 and 195-196 are pending in the instant application. Claims 18, 80, 89, 111, 121, 138, 149, 160, 175 and 191-194 have been cancelled without prejudice or disclaimer. Claims 1, 17, 81, 90, 101, 102, 110, 122, 130-132, 137, 139, 148, 150, 159, 161, 174 and 182 have been amended, and claims 195-196 have been added. Support for the claim amendments presented herein is found throughout the specification and in the claims as originally filed. Support for the aptamers and oligonucleotide transcripts having a length in the range of 30-50 nucleotides, as recited by amended claims 1, 101, 102 and 182, is found at least in paragraph **[0095]**, **[00100]** and **[00112]** of the specification as originally filed. Support for the specific combination of at least one 2'-OH guanosine, at least one 2'-OMe guanosine and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH), as recited by amended claims 1, 101, 102 and 182 and by new claims 195-196, is found at least in paragraphs **[00116]**, **[00122]** and **[00133]** and in Examples 1-2. Support for the amendments to claims 17, 110, 130-132, 137, 148, 159, and 174 is found at least in paragraphs **[0037]**, **[0056]**, **[0077]**, **[00121]**, **[00122]**, **[00146]**, and **[00213]**. Claims 81, 90, 122, 139, 150 and 161 have been amended solely to maintain claim dependency throughout the amended claim set. Accordingly, the present amendments are fully supported, and no new matter has been added.

Declaration Under 37 C.F.R. §1.132

The Examiner has objected to the Declaration of Dr. Anthony Keefe under 37 C.F.R. §1.132 ("the Declaration") that was submitted on September 30, 2008 in the instant application. The Examiner has asked for clarification regarding the axes on the graph presented in Exhibit A of the Declaration. The Examiner has also concluded that the totality of the rebuttal evidence in the Declaration fails to outweigh the evidence of obviousness.

The data presented in the graph in Exhibit A of the Declaration were generated using the commercially available ImageQuant™ software, as described in paragraph 7 of the Declaration. The values on the y-axis of the graph are arbitrary densitometry units that are generated by the

ImageQuant™ software and are used to quantify the level of radioactivity in a given gel band as detected by the phosphoroimager. In the graph shown in Exhibit A, the arbitrary densitometry unit values shown on the y-axis were normalized by subtracting the background signal levels detected by the phosphoroimager. The percentage values above each bar are the relative percentage transcription yield of each of the transcription reactions described in paragraphs 6 and 7 of the Declaration. The numbers on the X-axis of the graph represent the amount of $MgCl_2$ and/or $MnCl_2$ (in mM) in each reaction that was run. As shown in paragraph 6 of the Declaration, the total amount of divalent ions ($MgCl_2$ and $MnCl_2$) in each reaction was 8.5 nM, but the ratio of $MnCl_2$ to $MgCl_2$ was varied for each reaction. For example, in the first lane of the graph in Example A, 8.5 mM $MnCl_2$ and 0 nM $MgCl_2$ were added to the transcription reaction, and in the second lane, 7.5 mM $MnCl_2$ and 1 mM $MgCl_2$ were added to the transcription reaction, and so on.

Applicants submit that the Examiner's reliance on whether the presence of both magnesium and manganese ions is "specific or unexpected" with regard to transcription reactions that contain 2'-OMe NTP including 2'-OMe GTP is misplaced. The methods provided herein were carefully selected to produce efficient manufacturing processes that generate stabilized oligonucleotide transcripts and/or aptamers in a commercially effective manner. The evidence presented in the Declaration demonstrates that varying the concentration of magnesium and manganese ions can dramatically affect transcription yield. As discussed in more detail below in connection with the rejections under 35 U.S.C. § 103, the effect(s) of varying the ion concentrations on transcription could not have been predicted based on the teachings in the art at the time of instant invention. Thus, the identification of the ideal ratio of manganese and magnesium ions is not a "mere optimization" of ranges, nor would the ideal ratio have been obvious in view of the state of the art at the time of the instant invention.

The evidence set forth in the Declaration filed September 30, 2008 cannot be considered solely for its knockdown ability, but must be objectively considered, as set forth in *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143, (CCPA 1976):

> When prima facie obviousness is established and evidence is submitted in rebuttal, the decision-maker must start over. Though the burden of going forward to rebut the prima facie case remains with the applicant, the question of whether that burden has been successfully carried requires that the entire path to decision be retraced. An earlier decision should not, as it was here, be considered as set in

> concrete, and applicant's rebuttal evidence then be evaluated only on its knockdown ability. Analytical fixation on an earlier decision can tend to provide that decision with an undeservedly broadened umbrella effect. Prima facie obviousness is a legal conclusion, not a fact. Facts established by rebuttal evidence must be evaluated along with the facts on which the earlier conclusion was reached, not against the conclusion itself. Though the tribunal must begin anew, a final finding of obviousness may of course be reached, but such finding will rest upon evaluation of all facts in evidence, uninfluenced by any earlier conclusion reached by an earlier board upon a different record.

Applicants, therefore, respectfully request that the Examiner reconsider the rebuttal evidence provided in the Declaration submitted on September 30, 2008, which demonstrates the importance of using both magnesium and manganese ions in specific concentration ratios within the reaction conditions recited by the amended claims to allow the modified RNA polymerase to incorporate 2'-OMe NTPs.

Claim Rejections Under 35 U.S.C. § 112

Claims 1, 5-12, 14-20, 77-85, 88-94, 101-114, 116-127 and 130-194 have been rejected under 35 U.S.C. § 112, second paragraph for failing to particularly point out and distinctly claim the subject matter that Applicants regard as the invention. In particular, claims 1, 101, 102, 182 and 191-194 have been rejected as being unclear for reciting "comprising ... 2'-OMe guanosine ... including at least one 2'-OMe guanosine." Claims 17, 18, 89, 110, 111, 121, 138, 149, 160 and 175 have been rejected as being indefinite because "the term 'guanosine' ... is used by the claims to mean 'guanosine and/or monophosphate guanosine." (Office Action, pages 5-6).

Independent claims 1 and 101 and dependent claims 191-192 have been amended to recite stabilized aptamers that include at least one 2'-OH guanosine, at least one 2'-OMe guanosine and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH), while independent claims 102 and 182 and dependent claims 193-194 have been amended to recite stabilized oligonucleotide transcripts that include at least one 2'-OH guanosine, at least one 2'-OMe guanosine and at least one of 2'-OMe adenosine, 2'-OMe cytidine or 2'-OMe uridine (r/mGmH). Applicants submit that these amended claims are clear and definite. As such, withdrawal of this rejection is requested.

Claims 17, 110, 130-132, 137, 148, 159, and 174 have been amended to recite that the transcription reaction mixtures recited by the independent claims further include GMP.

Applicants submit that these amended claims are clear and definite. The Examiner should, therefore, withdraw this rejection.

Claim Rejections Under 35 U.S.C. § 103

Claims 1, 5-12, 14-17, 19-20, 77-79, 81-85, 88-94, 101-110, 112-114, 116-120, 122-127, 130-159, 161-174 and 176-194 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 5,660,985 by Pieken et al. ("Pieken") in view of Brieba et al., Biochemistry, vol. 39:919-923 (2000) ("Brieba"), U.S. Patent No. 6,107,037 by Sousa et al. ("Sousa"), and Bishop et al., J. Virol., vol. 8(1):66-73 (1971) ("Bishop"). Claims 18, 80, 89, 111, 121, 138, 149, 160 and 175 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Pieken in view Brieba, Sousa, Bishop and Milligan et al., Methods Enzymol., vol. 180: 51-62 (1989) ("Milligan").

Independent claims 1 and 101 have been amended to recite methods for identifying stabilized aptamers using specific transcription reaction conditions including the use of both magnesium and manganese ions to prepare mixtures of stabilized single-stranded nucleic acids having a length in the range of 30-50 nucleotides. Independent claims 102 and 182 have been amended to recite methods for transcribing stabilized oligonucleotide transcripts using specific transcription reaction conditions that include both magnesium and manganese ions to prepare mixtures of stabilized single-stranded nucleic acids having a length in the range of 30-50 nucleotides. The claimed methods produce stabilized nucleic acid molecules of a sufficient length for use in the SELEX process for identifying stabilized aptamers.

The cited references, alone or in combination, do not disclose or suggest the claimed methods. In fact, no combination of these references describes methods that use a modified RNA polymerase that can incorporate 2'-OMe NTPs in conjunction with a transcription reaction mixture that includes both magnesium and manganese ions to successfully produce stabilized nucleic acids having a length in the range of 30-50 nucleotides.

The Pieken reference describes only wild-type T7 RNA polymerases and does not disclose any transcription reaction mixtures that include both magnesium and manganese ions. The Pieken reference also explicitly acknowledges that the wild-type T7 RNA polymerase does not recognize bulkier 2'-substituted NTPs, such as 2'-OMe. (*See* Pieken, col. 8, lines 26-29).

Brieba does not disclose any modified RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs. Rather, this reference only describes the ability of a Y639/H784 double-mutant T7 RNA polymerase to incorporate NTPs with substituents capable of acting as hydrogen bond donors or acceptors (*e.g.*, 2'-OH, and 2'-NH_2) or NTPs with 2'-fluoro substituents into oligonucleotide transcripts. Moreover, this reference does not disclose a transcription reaction mixture that includes both magnesium and manganese ions, as acknowledged by the Examiner on page 10 of the Office Action. Thus, the skilled artisan seeking to produce stabilized nucleic acid molecules by incorporating 2'-OMe NTPs within an oligonucleotide transcript using a modified RNA polymerase would have no objective reason to turn to the Brieba reference.

Sousa does not teach or suggest any modified RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs into oligonucleotide transcripts, nor does this reference disclose the use of both magnesium and manganese ions in a single transcription reaction mixture. The Examiner has mischaracterized the teachings of the Sousa reference -- Sousa describes the use of manganese or magnesium ions in the transcription reaction buffer, but does not teach the use of both types of ions in a single buffer or transcription reaction. The passage cited by the Examiner at column 15, lines 44-48 describes two separate reaction conditions: one that was run using a buffer that contains magnesium ions and a second reaction that was run using a buffer with manganese ions:

> Transcription reactions: Transcription reactions were carried out in 40 mM Tris-Cl pH 8.0, 15 MM $MgCl_2$, and 5 mM DTT **or** 20 mM Manganese Citrate pH 8.0, 5 mM DTT at 37° C. (Emphasis added)

Moreover, Sousa teaches away from using manganese ions in the transcription reaction mixture by demonstrating "a sharp reduction in overall [polymerase] activity with Mn++ that was seen over a wide range of Mn++ concentrations that were tested in an effort to identify an optimal Mn++ concentration." (*See* Sousa, col. 22, lines 34-50). Accordingly, the skilled artisan seeking to use a modified RNA polymerase to produce stabilized nucleic acid molecules by incorporating 2'-OMe NTPs within an oligonucleotide transcript would have no objective reason to turn to the Sousa reference.

Milligan does not teach or suggest the use of any modified RNA polymerases, let alone modified RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs. Moreover, this reference does not disclose the use of both magnesium and manganese ions in a single transcription reaction mixture. In the passage cited by the Examiner, Milligan is concerned with conserving "valuable NTPs," such as modified or radiolabeled NTPs, by reducing the concentration of the valuable NTP in a transcription reaction mixture to concentrations as low as 50-100 μM. (*See* Milligan, pp. 58-59). This reference only teaches the use of GMP as a primer "if low concentrations of GTP are to be used." (*See* Milligan, p. 59; emphasis added). The transcription reaction conditions recited by the amended claims, however, do not require the use of low concentrations of 2'-OMe GTP. There is no objective reason in the teaching of Milligan that would indicate to the skilled artisan that the addition of GMP to a transcription reaction would be useful in situations other than when low concentrations of GTP are used. Accordingly, the skilled artisan seeking to identify transcription reaction conditions under which a modified polymerase could incorporate a 2'-OMe NTPs within an oligonucleotide transcript would not have been motivated to turn to the Milligan reference.

The addition of the Bishop reference in this case fails to remedy the deficiencies in the Pieken, Brieba, Sousa and/or Milligan references. Bishop does not teach or suggest the use of any modified RNA polymerases, let alone modified RNA polymerases that can incorporate bulkier 2'-modified NTPs such as 2'-OMe NTPs. Rather, the Bishop reference only describes the transcription reaction conditions for one particular unmodified (*i.e.*, wild-type) RNA polymerase.

The Abstract of Bishop clearly indicates that the authors have identified a specific set of *in vitro* reaction conditions for the maximal synthesis of RNA by one particular viral RNA polymerase. Bishop emphasizes that the conditions presented therein are specific for the influenza (WSN) virion ribonucleic acid polymerase. In the second paragraph of col. 1 on page 66, Bishop teaches that two different virus polymerases -- the influenza virus polymerase and the vesicular stomatitis virus (VSV) polymerase -- exhibit "distinct differences for the basic requirements of the two polymerases." Bishop demonstrates, *e.g.*, in Figure 2 and at page 68, col. 1, second full paragraph, that for one type of viral polymerase, the VSV polymerase, increasing levels of manganese ions actually inhibited the polymerase reaction.

Thus, contrary to the Examiner's assertions, Bishop does not teach the combination of magnesium and manganese ions is generally useful for optimal polymerase performance. In contrast, this reference is evidence that even within a single class of polymerases, *e.g.*, viral RNA polymerases, each individual polymerase can act in a distinctly different manner under the same conditions, and furthermore, each polymerase has an individual set of basic requirements for (i) initiating transcription generally and (ii) reaching a maximal level transcription. As such, transcription conditions that are useful for one type of polymerase cannot be used to predict the transcription conditions that will be needed for another polymerase to successfully and efficiently produce nucleic acid transcripts.

Accordingly, there is no teaching or other objective evidence that the conditions described in the Bishop reference would be broadly applicable to other types of non-viral RNA polymerases. Thus, there is no reason that the skilled artisan seeking to use a modified RNA polymerase to produce stabilized nucleic acid molecules by incorporating 2'-OMe NTPs within an oligonucleotide transcript would look to Bishop for teachings about polymerases in general, let alone for guidance regarding the conditions that would be necessary and sufficient to allow a modified polymerase to incorporate bulkier 2' substituents into oligonucleotide transcripts.

Applicants again submit that the Examiner has failed to establish a *prima facie* case of obviousness in the instant application. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one ordinary skill in the art. *See* MPEP §2143.01, citing *KSR International Co. v. Teleflex Inc.*, 550 U.S. 398, 82 USPQ2d 1385, 1396 (2007). Furthermore, a statement that modifications of the prior art to meet the claimed invention would have been "well within the ordinary skill of the art at the time the claimed invention was made" because the references relied upon teach that all aspects of the claimed invention were individually known in the art is not sufficient to establish a *prima facie* case of obviousness without some objective reason to combine the teachings of the references. *See* MPEP §2143.01, citing *Ex parte Levengood*, 28 USPQ2d 1300 (Bd. Pat. App. & Inter. 1993).

The Examiner has failed to present a convincing line of reasoning as to why the claimed methods and transcription conditions, which successfully incorporate all 2'-OMe NTPs into nucleic acid transcripts, would have been predictable to the skilled artisan based on the teachings

in the cited references, alone or in combination.

As discussed above, the combined use of magnesium and manganese ions is not rendered obvious by the Bishop reference, or any other cited reference. The Examiner's reliance on whether the presence of both magnesium and manganese ions in transcription reaction conditions was known in the art at the time the instant application was filed is misplaced. The methods provided herein were carefully selected to produce efficient manufacturing processes that generate stabilized oligonucleotide transcripts and/or aptamers in a commercially effective manner. The transcription reaction mixture and conditions recited by the amended claims, particularly the use of both magnesium and manganese ions, allow the modified RNA polymerases to accept any of the 2'-OMe NTPs as substrates and incorporate these modified nucleotides into the transcript during both the initiation and elongation portions of transcription.

Prior to the methods disclosed in the instant application, the skilled artisan could not incorporate 2'-OMe GTP in an oligonucleotide transcript -- none of the cited references disclose methods in which a polymerase is able to incorporate 2'-OMe GTP into an oligonucleotide transcript. Those of ordinary skill in the art at the time of the instant invention would have recognized the difficulty in incorporating 2'-OMe GTP during oligonucleotide transcription. Applicants again draw the Examiner's attention to a publication from 2004 by Chelliserrykattil and Ellington in which they admitted that all of the modified T7 RNA polymerase variants tested could not incorporate 2'-OMe GTP, and, therefore, they could not produce an oligonucleotide transcript having 2'-OMe GTP incorporated therein. (*See e.g.*, Chelliserrykattil and Ellington, Nature Biotechnology, vol. 22(9): 1155-1160 (2004), in particular at p. 1157, col. 2, lines 1-3 and Figure 2b, concluding that "none of the polymerases showed activity with 2'-O-methyl GTP." Additional copy enclosed herewith). This continued failure of others in the field to generate oligonucleotide transcripts that include 2'-OMe GTP is further evidence that the transcription conditions recited in the amended claims presented herein were not obvious at the time of filing.

Moreover, the methods of the claimed invention are a significant improvement over prior methods of producing stabilized nucleic acid molecules, such as stabilized aptamers. The methods provided herein do not require post-translational modifications to produce stabilized aptamers. Prior to the instant invention, 2'-OMe NTPs were incorporated within a given aptamer sequence by replacing the 2'-substituent of a nucleotide with a 2'-OMe group, a process referred

to as "post-SELEX™ modification." Once the 2'-OMe substituent(s) was incorporated post-translationally, the skilled artisan would then have to reevaluate the post-translationally stabilized aptamer to determine whether the modification would negatively affect or otherwise impact binding of the stabilized aptamer to the target molecule.

In contrast, the methods of the invention generate oligonucleotides that (i) are already stabilized by virtue of including one or more 2'-OMe NTPs within the transcribed nucleotide sequence and (ii) are already known to bind the desired target because only the stabilized transcribed nucleotide sequences that bind to the target are retained in the claimed methods. Thus, the manufacturing methods provided herein, which require fewer steps and provide a commercially efficient and effective method for producing stabilized aptamers, are a significant improvement over previous methods of manufacturing stabilized oligonucleotides known in the art at the time of the instant invention.

Applicants submit that there is no objective reason or other evidence provided in the cited references, alone or in combination, that the success of the claimed methods and transcription conditions would have been predictable, particularly in light of the inability of others in the field, *e.g.*, Chelliserrykattil and Ellington, even after the filing of the instant application, to identify and successfully use a modified polymerase to incorporate all 2'-OMe NTPs, including 2'-OMe GTP, within stabilized aptamers and/or stabilized oligonucleotide transcripts. Thus, any suggestion that it would have been obvious to use the modified RNA polymerases, transcription reaction mixtures and conditions recited by the amended claims is an improper application of hindsight based on Applicants' disclosure in the instant application. Accordingly, Applicants submit that the Examiner has failed to establish a *prima facie* case of obviousness and request that these rejections be withdrawn.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,

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